CAN HEME–CO COMPLEXES ALWAYS BE PHOTODISSOCIATED?

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1. Introduction

Yonetani et al. reported that the CO complexes of hemoproteins such as hemoglobin (Hb), myoglobin (Mb), horse radish peroxidase and yeast cytochrome *c* peroxidase release CO irreversibly by flash photolysis at 4.2°K and that one-half recombination occurs at 25–30°K [1]. On the other hand, cytochrome oxidase–CO is photolyzed irreversibly even at 77°K and the midpoint temperature for recombination is at 180°K [1]. When the intrinsic copper in cytochrome oxidase is removed by treating the oxidase with *p*-chloromercuribenzoate (*p*CMB) in the presence of sodium dodecyl sulfate (SDS) [2], however, its CO complex becomes insensitive to flash photolysis at 5°K [3]. A CO complex of free heme *a* is not photolyzed at this temperature either [3], in contrast with the report of Yonetani et al. that the photodissociation and recombination of the CO complexes of protoheme and heme *a* occur in the temperature range of 4.2–50°K. Therefore, in the present study we re-examined the spectral changes of these CO complexes upon irradiation under a variety of conditions but failed to confirm the photodissociation at 5°K, although recently there appeared some reports on the photodissociation of protoheme–CO over a wide temperature range from 5–340°K [4] and of CO complexes of protoheme–imidazole [5] and protoheme–mercaptide [6] at room temperatures. In order to reconcile these conflicting data, we would like to propose the mechanism underlying these phenomena, taking the effects of the apoprotein moiety and temperature on the strength of the heme iron–CO bond into consideration.

2. Materials and methods

Heme *a* was isolated from purified bovine cytochrome oxidase according to the method of Takemori and King [7] and dissolved in 1% (w/v) Na_2CO_3 aqueous solution. Protohemin (Sigma, type 1) was dissolved in 1 N NaOH or 1% Na_2CO_3 and used within a few hours after the preparation. In order to disperse heme *a* and protoheme aggregates in 0.05 M Na-phosphate buffer (pH 7.4) or in 0.05 M Tris–HCl (pH 7.5), either SDS or cetyltrimethylammonium bromide (CTAB) was added to a final concentration of 2.0% or 2.5%, respectively.

Picket fence heme was kindly supplied by Dr H. Hori of the Department of Biophysics, Osaka University, who synthesized it according to the method of Collman et al. [8].

SDS, CTAB, imidazole and pyridine and other chemicals were of the analytical grade and used without further purification.

The absorption spectra at cryogenic temperatures were measured on the instrument constructed by Hagihara and Iizuka [9] and equipped with a flash unit accessory [10].

3. Results and discussion

The difference absorption spectra of CO complexes of protoheme and heme *a* before (solid lines) and after (broken lines) firing of 20 flashes are illustrated in fig.1.

Usually the spectral change ceased with less than 10 flashes. Each difference spectrum after the irradiation reverted to a corresponding one before the irradiation as the sample temperature was raised to around 40°K. A CO complex of picket fence heme in 2.5% CTAB–pyridine behaved similarly. All of the difference spectrum after the irradiation, however,

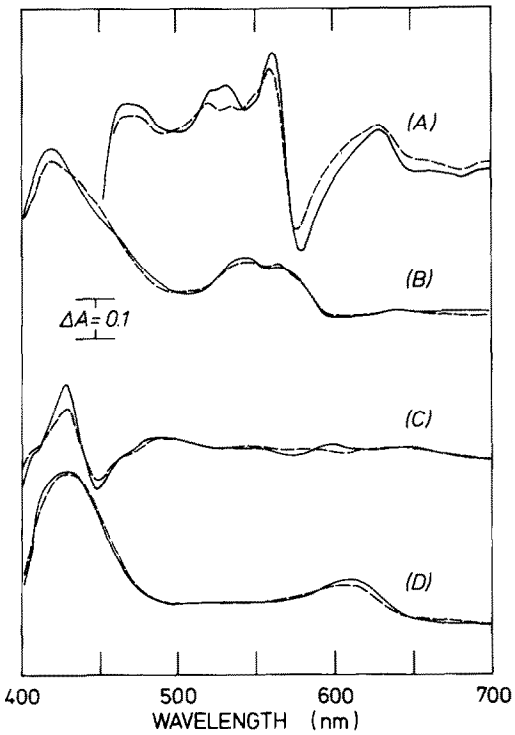

Fig.1. Difference spectra between heme–CO complex and the reduced heme before (solid line) and after (broken line) firing of 20 flashes at 5°K. A heme–CO complex was prepared by passing CO gas through a solution of the sample reduced with sodium dithionite, and placed in a sample compartment of the twin cuvette assembly. The reduced heme before the CO bubbling was placed in a reference compartment of the twin cuvette. (A) Protoheme (35.0 μM) in 2.5% CTAB, 0.05 M Tris–HCl buffer, pH 7.5. (B) Protoheme (20.5 μM) in 0.1 N NaOH (dimerized). (C) Heme *a* (18.1 μM) in 2% SDS, 0.05 M Na-phosphate buffer, pH 7.4 (monomerized). (D) Heme *a* (18.1 μM) in 0.05 M Na-phosphate buffer, pH 7.4 (aggregated).

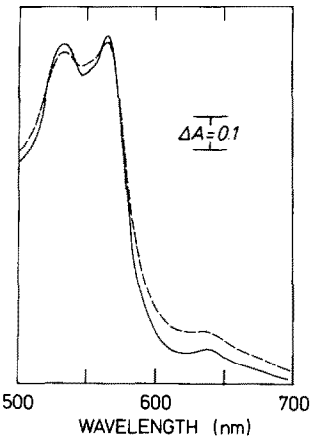

Fig.2. Absorption spectra of protoheme–CO before (solid line) and after (broken line) firing of 20 flashes at 5°K. Protohemin (29.6 μM) in 2.1 M pyridine, 0.075 N NaOH was reduced with sodium dithionite. The spectra were recorded with this medium as the reference. The α- and β-bands were at 565 nm and 533 nm, respectively, at 5°K.

were far from flat and retained the characteristics for the difference spectrum between the CO complex and its unligated form in each case. These results can be explained on the basis either that the heme–CO complexes are not photodissociated or that the recombination of photodissociated CO occurs so rapidly even at 5°K. The latter possibility is less likely, since the rate and extent of recombination, if it occurs, are too low as observed by Alberding et al. [4] in their recombination studies of protoheme–CO at very low temperatures.

Figure 2 illustrates the absolute absorption spectra for a CO complex of protoheme–pyridine almost invariable before and after flashing at 5°K, again militating against the photodissociation of CO. We confirmed that CO complexes of Mb, Hb A and its β-chain were evidently photodissociated at the temperature of liquid helium under the same instrumental conditions, so that the small spectral changes of heme–CO upon irradiation are attributable to their inherent properties. It is highly possible, therefore, that these changes are due to photoexcitation which brings about different modes of ligand binding or distortion of the porphyrin nucleus without accompanying the photodissociation. In fact, the broadening of the absorption band of coordination compounds on photoexcitation has been reported and discussed

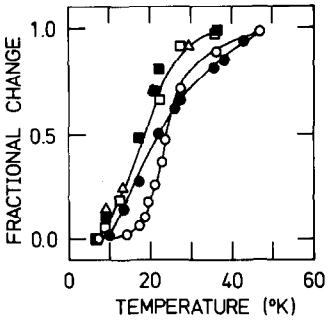

Fig.3. Temperature-dependent restoration of the absorbance change induced by irradiation at 5°K. The absorbance change was monitored at 764 nm for Hb A, and for other heme–CO complexes at wavelengths where the largest change occurred in the visible region. The rate of the temperature rise was almost the same in each case. (–○–) Hb A in 0.05 M Tris–HCl buffer, pH 7.5. (–●–) Protoheme in 2.1 M pyridine, 0.075 N NaOH. (–■–) Protoheme in 2.5% CTAB, 0.05 M Tris–HCl buffer, pH 7.5. (–△–) Heme *a* in 2% SDS, 0.05 M Na-phosphate buffer, pH 7.4. (–□–) Picket fence heme in 2.5% CTAB, 2.1 M pyridine.

theoretically by Balzani and Carassiti [11]. This broadening effect might account for a rather flat line in the visible region observed upon irradiation of heme *a*–CO in 2% SDS (fig.1, line c).

The absorbance change induced by irradiating the CO complexes at 5°K was restored, irrespective of its nature, as the sample temperature was raised (fig.3). It is noteworthy that the recombination of the photodissociated CO to Hb and the deactivation of photoexcited heme–CO complexes apparently gave the similar profiles. In this connection, although Yonetani et al. already gave the same profile for protoheme–CO (fig.2 in ref. [1]) as the present one regarding this as the recombination process, we would guess this actually being the deactivation.

Alberding et al. extensively studied the dynamics of CO binding to protoheme at temperatures from 5–340°K. According to them, the extent of absorbance changes at 425 nm observed upon irradiation at 230°K was 1/100 that observed at 290°K (fig.1 in ref. [4]) and as the temperature further went down to 20°K this extent increased again approaching 1/3 that at 290°K. Although they regarded these absorbance changes at 290°K and 20°K as being caused equally by photodissociation, it is also highly possible, based on our present results, that the absorbance change they observed in the low temperature range is ascribed to photoexcitation rather than photodissociation. On the contrary, the absorbance change in the high temperature range would be ascribed to the photodissociation of a CO complex of protoheme–imidazole as observed by Chang and Traylor [5]. Accordingly, it is suggested that heme–CO complexes are not always photodissociable and that they become insensitive to irradiation especially at the temperature of liquid helium.

In order to explain the negligible photodissociability of a heme *a*–CO complex at this temperature, Orii et al. [3] supposed, based on a proposal made by Kitagawa et al. [12], that in this complex the bond axis between the C and O atoms would be perpendicular to the heme plane in favor of a σ-bond, and that this arrangement would render the complex less photodissociable. This explanation seemingly contradicts with the photodissociation of protoheme–CO complexes observed at room temperatures [4,5], in which the Fe–C–O arrangement may be linear as indicated by the X-ray crystallographic study for a CO complex of an iron (II) porphyrin [13]. However, it is conceivable that at room temperatures collisions of solvent molecules with the ligated CO increases the chance for the C–O bond to tilt towards the heme plane, whereas that at very low temperatures the CO molecule stays at the upright position. Hence, at room temperature the empty π orbital of the CO molecule can combine easily with the iron d_π-orbital forming a back bonding, a prerequisite for the formation of the antibonding orbital with respect to the Fe–CO bond [12] and consequently the complex becomes photodissociable. In hemoprotein–CO complexes the apoprotein moiety would bend the iron–CO bond, as proposed for cytochrome oxidase–CO [3], making the bond photodissociable. Thus, we propose that the temperature and apoprotein moiety determine the nature and strength of the bonding of CO to heme iron and hence the photodissociability. Experiments are in progress to prove this hypothesis.

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